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DOCUMENT-IDENTIFIER: US 6204363 B1
TITLE: Stem cell factor

BSPR:
It is believed that small amounts of certain hematopoietic growth factors account for the differentiation of a small number of "stem cells" into a variety of blood cell progenitors for the tremendous proliferation of those cells, and for the ultimate differentiation of mature blood cells from those lines. The hematopoietic regenerative system functions well under normal conditions. However, when stressed by chemotherapy, radiation, or natural myelodysplastic disorders, a resulting period during which patients are seriously leukopenic, anemic, or thrombocytopenic occurs. The development and the use of hematopoietic growth factors accelerates bone marrow regeneration during this dangerous phase.

BSPR:
Because the hematopoietic growth factors are present in extremely small amounts, the detection and identification of these factors has relied upon an array of assays which as yet only distinguish among the different factors on the basis of stimulative effects on cultured cells under artificial conditions.

BSPR:
The application of recombinant genetic techniques has clarified the understanding of the biological activities of individual growth factors. For example, the amino acid and DNA sequences for human erythropoietin (EPO), which stimulates the production of erythrocytes, have been obtained. (See, Lin, U.S. Pat. No. 4,703,008, hereby incorporated by reference). Recombinant methods have also been applied to the isolation of cDNA for a human granulocyte colony-stimulating factor, G-CSF (See, Souza, U.S. Pat. No. 4,810,643, hereby incorporated by reference), and human granulocyte-macrophage colony stimulating factor (GM-CSF) [Lee, et al., Proc. Natl. Acad. Sci. USA, 82, 4360-4364 (1985); Wong, et al., Science, 228, 810-814 (1985)], murine G- and GM-CSF [Yokota, et al., Proc. Natl. Acad. Sci. (USA), 81, 1070 (1984); Fung, et al., Nature, 307, 233 (1984); Gough, et al., Nature, 309, 763 (1984)], and human macrophage colony-stimulating factor (CSF-1) [Kawasaki, et al., Science, 230, 291 (1985)].

BSPR:

An activity in human spleen conditioned medium has been termed synergistic factor (SP). Several human tissues and human and mouse cell lines produce an SF, referred to as SF-1, which synergizes with CSF-1 to stimulate the earliest HPP-CFC. SF-1 has been reported in media conditioned by human spleen cells, human placental cells, 5637 cells (a bladder carcinoma cell line), and EMT-6 cells (a mouse mammary carcinoma cell line). The identity of SF-1 has yet to be determined. Initial reports demonstrate overlapping activities of interleukin-1 with SF-1 from cell line 5637 [Zsebo et al., Blood, 71, 962-968 (1988)]. However, additional reports have demonstrated that the combination of interleukin-1 (IL-1) plus CSF-1 cannot stimulate the same colony formation as can be obtained with CSF-1 plus partially purified preparations of 5637 conditioned media [McNiece, Blood, 73, 919 (1989)].

BSPR:
Another class of synergistic factor has been shown to be present in conditioned media from TC-1 cells (bone marrow-derived stromal cells). This cell line produces a factor which stimulates both early myeloid and lymphoid cell types. It has been termed hemolymphopoietic growth factor 1 (HLGF-1). It has an apparent molecular weight of 120,000 [McNiece et al., Exp. Hematol., 16, 383 (1988)].

DEPR:
There is embryonic expression of SCF by cells in the migratory pathway and homing sites of melanoblasts, germ cells, hematopoietic cells, brain and spinal chord.

DEPR:
The biological activity and pattern of tissue distribution of SCF demonstrates its central role in embryogenesis and hematopoiesis as well as its capacity for treatment of various stem cell deficiencies.

DEPR:
Also comprehended by the invention are pharmaceutical compositions comprising therapeutically effective amounts of polypeptide products of the invention together with suitable diluents, preservatives, solubilizers, emulsifiers, adjuvants and/or carriers useful in SCF therapy. A "therapeutically effective amount" as used herein refers to that amount which provides a therapeutic effect for a given condition and administration regimen. Such compositions are liquids or lyophilized or otherwise dried formulations and include diluents of various buffer content (e.g., Tris-HCl., acetate, phosphate), pH and ionic strength, additives such as albumin or gelatin to prevent adsorption to surfaces, detergents (e.g., Tween 20, Tween 80, Pluronic F68, bile acid salts), solubilizing agents (e.g., glycerol, polyethylene glycol), anti-oxidants (e.g., ascorbic acid, sodium metabisulfite), preservatives (e.g., Thimerosal, benzyl alcohol, parabens), bulking substances or tonicity modifiers (e.g., lactose, mannitol), covalent attachment of polymers such as polyethylene glycol to the protein (described in Example 12 below), complexation with metal ions, or incorporation of the material

into or onto particulate preparations of polymeric compounds such as polylactic acid, polglycolic acid, hydrogels, etc. or into liposomes, microemulsions, micelles, unilamellar or multilamellar vesicles, erythrocyte ghosts, or spheroplasts. Such compositions will influence the physical state, solubility, stability, rate of in vivo release, and rate of in vivo clearance of SCF. The choice of composition will depend on the physical and chemical properties of the protein having SCF activity. For example, a product derived from a membrane-bound form of SCF may require a formulation containing detergent. Controlled or sustained release compositions include formulation in lipophilic depots (e.g., fatty acids, waxes, oils). Also comprehended by the invention are particulate compositions coated with polymers (e.g., poloxamers or poloxamines) and SCF coupled to antibodies directed against tissue-specific receptors, ligands or antigens or coupled to ligands of tissue-specific receptors. Other embodiments of the compositions of the invention incorporate particulate forms, protective coatings, protease inhibitors or permeation enhancers for various routes of administration, including parenteral, pulmonary, nasal and oral.

DEPR:
The invention also comprises compositions including one or more additional hematopoietic factors such as EPO, G-CSF, GM-CSF, CSF-1, IL-1, IL-2, IL-3, IL-4, IL-5, IL-6, IL-7, IL-8, IL-9, IL-10, IL-11, IGF-I, or LIF (Leukemic Inhibitory Factor).

DEPR:
Polypeptides of the invention may be "labeled" by association with a detectable marker substance (e.g., radiolabeled with .sup.125 I or biotinylated) to provide reagents useful in detection and quantification of SCF or its receptor bearing cells in solid tissue and fluid samples such as blood or urine.

DEPR:
SCF is useful, alone or in combination with other therapy, in the treatment of a number of hematopoietic disorders. SCF can be used alone or with one or more additional hematopoietic factors such as EPO, G-CSF, GM-CSF, CSF-1, IL-1, IL-2, IL-3, IL-4, IL-5, IL-6, IL-7, IL-8, IL-9, IL-10, IL-11, IL-1, IGF-I or LIF in the treatment of hematopoietic disorders.

DEPR:
There is a group of stem cell disorders which are characterized by a reduction in functional marrow mass due to toxic, radiant, or immunologic injury and which may be treatable with SCF. Aplastic anemia is a stem cell disorder in which there is a fatty replacement of hematopoietic tissue and pancytopenia. SCF enhances hematopoietic proliferation and is useful in treating aplastic anemia (Example 8B). Steel mice are used as a model of human aplastic anemia [Jones, Exp. Hematol., 11, 571-580 (1983)]. Promising results have been obtained with the use of a related cytokine, GM-CSF in the treatment of aplastic anemia [Antin, et al., Blood, 70, 129a (1987)]. Paroxysmal nocturnal hemoglobinuria (PNH) is a stem cell disorder characterized by

formation of defective platelets and granulocytes as well as abnormal erythrocytes.

DEPR:
Enhancement of growth in non-hematopoietic stem cells such as primordial germ cells, neural crest derived melanocytes, commissural axons originating from the dorsal spinal cord, crypt cells of the gut, mesonephric and metanephric kidney tubules, and olfactory bulbs is of benefit in states where specific tissue damage has occurred to these sites. SCF is useful for treating neurological damage and is a growth factor for nerve cells. SCF is useful during in vitro fertilization procedures or in treatment of infertility states. SCF is useful for treating intestinal damage resulting from irradiation or chemotherapy.

10/21/03 VLL Irene Shawman 10/21/03

DEPR:
There are numerous cases which document the increased proliferation of leukemic cells to the hematopoietic cell growth factors G-CSF, GM-CSF, and IL-3 [Delwel, et al., Blood, 72, 1944-1949 (1988)]. Since the success of many chemotherapeutic drugs depends on the fact that neoplastic cells cycle more actively than normal cells, SCF alone or in combination with other factors acts as a growth factor for neoplastic cells and sensitizes them to the toxic effects of chemotherapeutic drugs. The overexpression of SCF receptors on leukemic blasts is shown in Example 13.

DEPR:
A number of recombinant hematopoietic factors are undergoing investigation for their ability to shorten the leukocyte nadir resulting from chemotherapy and radiation regimens. Although these factors are very useful in this setting, there is an early hematopoietic compartment which is damaged, especially by radiation, and has to be repopulated before these later-acting growth factors can exert their optimal action. The use of SCF alone or in combination with these factors further shortens or eliminates the leukocyte and platelet nadir resulting from chemotherapy or radiation treatment. In addition, SCF allows for a dose intensification of the anti-neoplastic or irradiation regimen (Example 19).

DEPR:
SCF is useful for expanding early hematopoietic progenitors in syngeneic, allogeneic, or autologous bone marrow transplantation. The use of hematopoietic growth factors has been shown to decrease the time for neutrophil recovery after transplantation [Donahue, et al., Nature, 321, 872-875 (1986) and Welte et al., J. Exp. Med., 165, 941-948, (1987)]. For bone marrow transplantation, the following three scenarios are used alone or in combination: a donor is treated with SCF alone or in combination with other hematopoietic factors prior to bone marrow aspiration or peripheral blood leucophoresis to increase the number of cells available for transplantation; the bone marrow is treated in vitro to activate or expand the cell number prior to transplantation; finally, the recipient is treated to enhance engraftment of the donor marrow.

DEPR:
Another useful biological activity of both naturally-derived and recombinant rat SCF is the ability to cause the proliferation of the IL-4 dependent murine mast cell line, MC/9 (ATCC CRL 8306). MC/9 cells are cultured with a source of IL-4 according to the ATCC CRL 8306 protocol. The medium used in the bioassay is RPMI 1640, 4% fetal bovine serum, 5.times.10.sup.-5 M 2-mercaptoethanol, and 1.times.glutamine-pen-strep. The MC/9 cells proliferate in response to SCF without the requirement for other growth factors. This proliferation is measured by first culturing the cells for 24 h without growth factors, plating 5000 cells in each well of 96 well plates with test sample for 48 h, pulsing for 4 h with 0.5 uCi .sup.3 H-thymidine (specific activity 20 Ci/mmol), harvesting the solution onto glass fiber filters, and then measuring specifically-bound radioactivity. This assay was used in the purification of mammalian cell derived rat SCF after the ACA 54 gel filtration step, section C2 of this Example. Typically, SCF caused a 4-10 fold increase in CPM over background.

DEPR:
First strand cDNA was prepared from total RNA or poly A.sup.+ RNA from monkey liver (purchased from Clontech) and from the cell lines NIH-3T3 (mouse, ATCC CRL 1658), D17 (dog, ATCC CCL 183), bovine endothelial cell line (provided by Yves DeClerck, Childrens Hospital Los Angeles, Los Angeles, Calif.), feline embryonic fibroblast cell line (Jarrett et al., J. Gen. Virology, 20:169-175 (1973)) and chicken brain RNA. The primer used in first strand cDNA synthesis was either the nonspecific primer 228-28 or an SCF primer (227-30, 237-19, 237-20, 230-25 or 241-6).

DEPR:
For transient expression in COS-1 cells (ATCC CRL 1650), vector V19.8 (Example 3C) containing the rat SCF.sup.1-162 and SCF.sup.1-193 genes was transfected into duplicate 60 mm plates [Wigler et al., Cell, 14, 725-731 (1978)]. The plasmid V19.8 SCF is shown in FIG. 17. As a control, the vector without insert was also transfected. Tissue culture supernatants were harvested at various time points post-transfection and assayed for biological activity. Table 4 summarizes the HPP-CFC bioassay results and Table 5 summarizes the MC/9 .sup.3 H-thymidine uptake data from typical transfection experiments. Bioassay results of supernatants from COS-1 cells transfected with the following plasmids are shown in Tables 4 and 5: a C-terminally-truncated form of rat SCF with the C-terminus at amino acid position 162 (V19.8 rat SCF.sup.1-162), SCF.sup.1-162 containing a glutamic acid at position 81 [V19.8 rat SCF.sup.1-162 (Glu81)], and SCF.sup.1-162 containing an alanine at position 19 [V19.8 rat SCF.sup.1-162 (Ala19)]. The amino acid substitutions were the product of PCR reactions performed in the amplification of rat SCF.sup.1-162 as indicated in Example 3. Individual clones of V19.8 rat SCF.sup.1-162 were sequenced and two clones were found to have amino acid substitutions. As can be seen in Tables 4 and 5, the recombinant rat SCF (also referred to throughout this

application as rrat SCF or rrSCF), is active in the bioassays used to purify natural mammalian SCF in Example 1.

DEPR:
Recombinant human SCF was tested in agar colony assays on human bone marrow in combination with other growth factors as described above. The results are shown in Table 13. SCF synergizes with G-CSF, GM-CSF, IL-3, and EPO to increase the proliferation of bone marrow targets for the individual CSFs.

DEPR:
The ability of recombinant rat SCF.sup.1-164 (rrSCF.sup.1-164), to act synergistically with IL-7 to enhance lymphoid cell proliferation was studied in agar cultures of mouse bone marrow. In this assay, the colonies formed with rrSCF.sup.1-164 alone contained monocytes, neutrophils, and blast cells, while the colonies stimulated by IL-7 alone or in combination with rrSCF.sup.1-164 contained primarily pre-B cells. Pre-B cells, characterized as B220.sup.+, sIg.sup.-, c.mu..sup.+, were identified by FACS analysis of pooled cells using fluorescence-labeled antibodies to the B220 antigen (Coffman, Immunol. Rev., 69, 5 (1982)] and to surface Ig (FITC-goat anti-K, Southern Biotechnology Assoc., Birmingham, Ala.); and by analysis of cytospin slides for cytoplasmic .mu. expression using fluorescence-labeled antibodies (TRITC-goat anti-.mu., Southern Biotechnology Assoc.,). Recombinant human IL-7 (rhIL-7) was obtained from Biosource International (Westlake Village, Calif.). When rrSCF.sup.1-164 was added in combination with the pre-B cell growth factor IL-7, a synergistic increase in colony formation was observed (Table 16), indicating a stimulatory role of rrSCF.sup.1-164 on early B cell progenitors.

DEPR:
Lewis rats, male, weighing approximately 225 gms, were injected intravenously via the dorsal vein of the penis with either polyethylenesporeglycol-modified ratSCF-PEG (Examples 10 and 12), recombinant human G-CSF, a combination of both growth factors, or with carrier consisting of 1% normal rat serum in sterile saline. Quantitative peripheral blood and bone marrow differentials were performed at various timepoints as previously described [Hulse, Acta Haematol. 31:50 (1964); Chervenick et al., Am. J. Physiol. 215: 353 (1968)]. Histologic examination of the spleen was performed with Bouin's-fixed paraffin-embedded sections stained with hematoxylin-and-eosin as well as by the Giemsa method. The numbers of normoblasts, megakaryocytes, and mast cells per 400.times. or 1000.times. high power field (HPF) in the spleen was quantitated by counting the number of each cell type in randomly selected fields of the red pulp. Increases in circulating numbers of neutrophils over extended time periods were when so stated calculated by planimetry as previously described. [Ulich et al., Blood 75:48 (1990)]. Data is expressed as the mean plus-or-minus one standard deviation and statistical analysis is by the unpaired t-test.

DEPR:

These results demonstrate that the in vivo combination of ratSCF-PEG and G-CSF causes a synergistic myeloid hyperplasia in the bone marrow and spleen and a synergistic increase in circulating neutrophils. The synergism between a single dose of ratSCF-PEG and G-CSF becomes most dramatically apparent as a rapidly increasing number of circulating neutrophils between 4 and 6 hours after commencement of administration of growth factors. Daily coinjection plus G-CSF for one week causes a highly synergistic increase in circulating neutrophils as compared to ratSCF-PEG alone or G-CSF alone.

DEPR:
Though single factors such as G-CSF have been shown to have important effects on hematopoietic recovery, the combination of SCF with G-CSF has a dramatic hematologic response. In the first set of experiments, 3 normal dogs were treated with recombinant canine SCF alone at 200 .mu.g/kg/day subcutaneously or by continuous intravenous infusion. These animals responded with an increase in the white blood cell count to 30-50,000/mm3, from a baseline of 10-15,000 mm.sup.3 by day 8-12. When another group of normal dogs were treated for 28 days with recombinant canine SCF (200 .mu.g/kg/day SCF and G-CSF (10 .mu.g/kg/day SC), the white blood cell count increased from a normal range of 10-11,000/ mm.sup.3 to 200-240,000 cells/mm.sup.3 by day 17-21. This demonstrates that the effects of SCF are dramatically enhanced in combination with other hematopoietic growth factors. Similarly, in vitro data show that SCF in combination with EPO dramatically enhances BFU-E growth (number and size, see Example 9), again demonstrating that combinations of hematopoietic growth factors are more effective in eliciting a hematopoietic response and/or may allow for lower doses of other factors to elicit the same response.

DEPR:
As noted above, there are several ways that SCF is useful to improve hematopoietic transplantation. One method, as illustrated above is to use SCF to augment the harvest of bone marrow and/or peripheral blood progenitors and stem cells by pretreating the donor with SCF. Another use is to treat the recipient of the transplanted cells with SCF after the patient has been infused. The recipient is treated with SCF alone or in combination with other early and late acting recombinant hematopoietic growth factors, including EPO, G-CSF, GM-CSF, M-CSF, IL-1, IL-3, IL-6, etc.

DEPR:
SCF alone enhances hematopoietic recovery following bone marrow transplantation. A variety of experimental variables have been tested in a canine model of bone marrow transplantation, Schuening et al., 76 636-640. In one set of experiments for the present invention, dogs received either G-CSF or SCF after 920 cGy of total body irradiation and 4.times.108 mononuclear marrow cells per kilogram from a DLA-identical littermate. The hematologic recovery, as measured by day of neutrophil recovery to 500 or 1000/mm.sup.3, is accelerated when either SCF or G-CSF is administered compared to control animals that received

no growth factor (Table 21). Recovery was 2-6 days earlier in animals that received SCF than it was in those that received no growth factor. As noted above, combinations of appropriate growth factors with SCF will accelerate and enhance the response to those growth factors following hematopoietic transplantation.

DEPR:
Cyclic neutropenia, in particular, is a defect in the regulation of stem cell division since other lineages (e.g., platelet, erythrocyte and monocyte) are also effected. In the canine model of cyclic neutropenia, the cycling of neutrophils, as well as other lineages, is sharply reduced or even eliminated by SCF treatment. A typical dog with cyclic neutropenia was treated with rcanineSCF (recombinant canine SCF) at 100 mg/kg/day subcutaneously over several weeks. The typical 21 day cycle for neutrophils was eliminated during the first predicted cycle and the second predicted nadir was significantly atenuated. This is in contrast to treatment with G-CSF which increases the frequency and amplitude of neutrophil cycling, but does not eliminate it. Thus, SCF is useful in treating a variety of bone marrow failure syndromes, either alone or in combination with other hematopoietic growth factors.

DEPR:
The in vitro survival and proliferation of primitive stem cells is critical to the success of gene transfer mediated by retroviral insertion or other known methods of gene transfer. The effect of SCF on the in vitro maintenance and/or proliferation of primitive progenitor cells has been studied in two systems which have been described previously [Bodine et al., Proc. Natl. Acad. Sci. 86 8897-8901, 1989]. The first is a pre-CFU-S assay wherein bone marrow cells are incubated for up to six days in suspension culture in the presence of growth factors. Aliquots are injected into lethally iradiated mice and the mice sacrificed at 12-14 days for quantitation of spleen focus formation. IL-3 and IL-6 synergize in enhancing the proliferation of CFU-S between 2-6 days in culture. The second is a competitive repopulation assay which measures the effects of growth factors on recovery and biological activity of cells capable of sustaining long-term hematopoiesis. Cells from two congenic strains of mice differing for a hemoglobin marker are incubated in suspension independently, cells from one strain as a control and cells from a second under experimental conditions. After incubation, equal numbers of bone marrow cells from both cultures are mixed and injected into W/W.sup.v recipients.

DEPR:
In the competitive repopulation assay, the repopulating ability of cells cultured in the combination of SCF and IL-6 is superior at 35 days (short-term reconstitution) (FIG. 67). A most advantageous combination for long term reconstitution is SCF, IL-3 and IL-6, approximately 1.5-fold greater than any combination of two factors. Based on these data, a most advantageous combination of soluble growth factors for

enhancing retroviral mediated gene transfer into stem cells would be SCF, IL-3 and IL-6.

DEPL:
B. Synergistic Effect of SCF and Other Growth Factors in Canines.

DEPC:
Synergistic Effect of SCF and Other Growth Factors

ORPL:
Blood, A.B. Kriegler et al., "Partial purific. and characterization of a growth factor for macrophage progenitor cells with high proliferative potential in mouse bone marrow", vol. 60, pp. 503-508, Aug. 2, 1982.

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ORPL:
Abstract, Hollands, "Differentiation of embryonic haemopoietic stem cells from mouse blastocysts grown in vitro", vol. 102, pp. 135-141, Jan. 1988, DIALOG File 155 Accession No. 88328789 of Development.

ORPL:
British Medical Bulletin, Dexter, "Haemaopoeitic growth factors", vol. 45, pp. 337-349 Published 1989.

ORPL:
Abstract, Schrader, "Role of a single haemopoietic growth factor in multiple proliferative disorders of haemopoietic and related cells", vol. 2, pp. 133-137, Jul. 1983, DIALOG File 155 Acc. No. 84244875 of Lancet.